CLAIMS

1. A method of determining the nucleotide sequence of a polynucleotide, comprising the steps of:

a) cleaving the polynucleotide with a restriction enzyme so as to generate two or more fragments, wherein the restriction enzyme cleaves the polynucleotide at a site away from the restriction enzyme's recognition site so as to generate a cleaved site possessing a recessed 3'-end and a 5'-overhang of undefined sequence;

b) filling-in said recessed 3'-ends so as to form substantially blunt-ended fragments;

c) cloning and sequencing said blunt-ended fragments;

d) pairing matching blunt-ends of said blunt-ended fragments so as to allow said blunt-ended fragments to be ordered in a contiguous over-lapping arrangement; and

e) reading said nucleotide sequence from said contiguous arrangement.

2. The method according to claim 1 wherein the restriction enzyme generates a 5'-overhang of 3 or more bases in length.

3. The method according to claim 2 wherein the restriction enzyme is selected from *Hga*I, *Alw*26I, *Bbv*I, *Bsm*AI, *Bsm*FI, *Bst*71I, *Fok*I, *Sfa*NI, *Eam*1104I, *Ear*I, *Ksp*632I, *Bbs*I, *Bbv*16II, *Bpi*I, *Bpu*AI, *Bsa*I, *Eco*31I, *Bsm*B1, *Esp*3I, *Bsp*MI, *Gdi*II and/or *Sap*I.

4. The method according to any preceeding claim wherein the polynucleotide is cleaved with two or more of said restriction enzymes.

5. The method according to any preceeding claim wherein the recessed 3'-ends are filled in by employing a DNA polymerase and a mixture of deoxynucleotide triphosphates containing dATP, dCTP, dGTP and dTTP, so as to generate substantially blunt-ends.

6. The method according to claim 5 wherein the DNA polymerase is DNA polymerase I, *Pfu* polymerase, *Tli* polymerase, *Taq* polymerase, *Tfl* polymerase or *Tth* polymerase.

7. The method according to any proceeding claim wherein the blunt-ended fragments possess a single adenine 3'-overhang and the cloning of said fragments is facilitated using a cleaved vector comprising single thymidine 5'-overhangs at the cleavage site.

8. The method according to any preceeding claim wherein the pairing of the matching ends and ordering of the fragments into a contiguous over-lapping arrangement is carried out by using a computer program designed for such an application.

9. The method according to any preceeding claim wherein reading of said nucleotide sequence from said contiguous arrangement is carried out by or with the assistance of a computer.

10. The method according to any one of claims 1 to 8 for use in conducting restriction mapping of a polynucleotide.

11. A computer program for use with the method according to any preceeding claim wherein the computer program serves to pair the matching ends of the sequenced fragments and order the fragments into a contiguous overlapping arrangement.

12. The computer program according to claim 11 which further reads from the contiguous overlapping arrangement and provides the user with the nucleotide sequence of the polynucleotide.

13. An semi-automated or fully automated sequencing apparatus with a dedicated computer comprising the computer program according to either of claims 11 or 12.

14. A kit suitable for use with the method according to any one of claims 1 to 10 wherein the kit comprises at least one of said restriction enzymes and a DNA polymerase(s) for the filling-in and/or sequencing reactions.

15. A kit according to claim 14 further comprising a vector for cloning the substantially blunt-ended fragments.

16. A kit according to claim 15 wherein the vector is a cleaved vector comprising single thymidine 5'-overhangs at the cleavage site.

17. A kit according to claim 16 wherein the vector is a pGEM®-T vector or a TA Cloning® Vector.

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18. A kit according to any one of claims 14 to 17 further comprising a computer program according to either of claims 11 or 12 in machine readable form.